Synthesis of Two Potential Inhibitors of *para*-Aminobenzoic Acid Synthase

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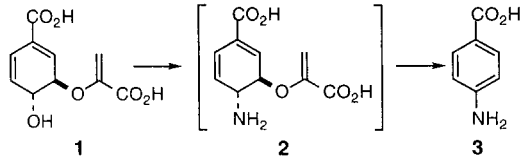
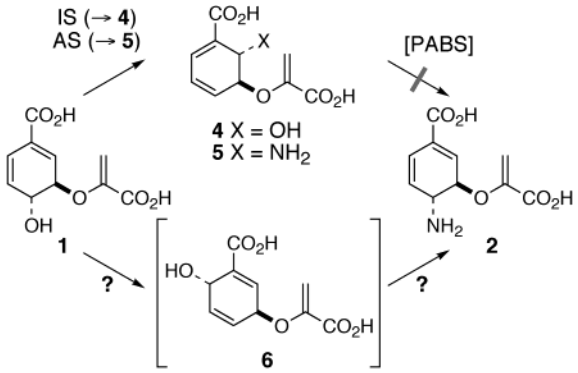
The shikimate−chorismate pathway is the biosynthetic sequence that leads from carbohydrates to aromatic metabolites in plants, bacteria, and fungi.[1] Since this metabolic pathway is not present in mammals, it has been an attractive target for the development of herbicides[2] and antibacterial agents.[3] Recent studies have indicated that some parasites may also contain portions of the shikimate pathway.[4] In addition, many of the enzymes in the sequence involve unusual mechanisms and provide interesting challenges for the design of inhibitors. The focus of this report is the synthesis of two proposed inhibitors of *para*-aminobenzoic acid synthase (PABS), a branchpoint enzyme occurring late in the pathway. PABS is responsible for the transformation of chorismic acid, **1**, to aminodeoxychorismate, **2** (ADC), and then by elimination to *para*-aminobenzoic acid, **3** (PABA). The enzyme comprises three components:[5,6] PabA is a glutaminase that furnishes the ammonia, PabB effects the conversion of **1** to **2**, and PabC eliminates pyruvate from **2** to give PABA itself.



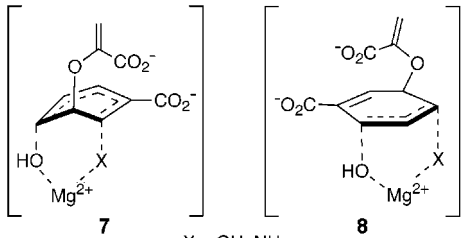
Isochorismate synthase (IS) and the isomerase component of anthranilate synthase (AS) also catalyze transformations of chorismate in which the hydroxyl group at C-4 is isomerized and replaced, to give **4** and **5**, respectively. Although there is considerable sequence homology between these proteins and PabB, the nature of the substitution reaction itself, as well as previous inhibition results,[7] suggests that there are significant mechanistic differences between the PabB transformation and those of IS and AS.

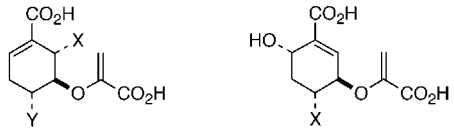
Several potential mechanisms for PabB have been suggested to explain direct substitution at C-4 with



retention of configuration. Two observations argue against a mechanism involving initial isomerization to isochorismic acid, **4**, followed by displacement by ammonia in a back reaction: isochorismate is not a good alternative substrate for PABS,[6,8] and the enzyme is only poorly inhibited by analogues that bind with high affinity to IS and AS.[7] Mattia and Ganem have proposed a different intermediate, **6**, that would be formed and then converted to ADC by a mechanism closely related to that favored for IS and AS.[9] They synthesized this compound, but the rapidity with which it aromatizes and rearranges precluded its assessment as a substrate for the enzyme.

The involvement of Mg^{2+} as a cofactor and the *syn*-stereochemistry of all of these processes are consistent with transition states such as **7** and **8**, in which the cation coordinates both the leaving and attacking groups in S_N' and S_N'' substitutions.[10] As mimics of **7**, the diol and amino alcohol analogues **9**−**11** were shown to be potent inhibitors of IS and AS (but not PABS).[7] We now report the synthesis of the isomeric compounds, **12-O** and **12-N**, which mimic the transition states leading to and from the putative PabB intermediate **6**.





9: X = Y = OH
10: X = NH_2, Y = OH
11: X = OH, Y = NH_2

12-O: X = OH
12-N: X = NH_2

Key criteria for synthetic routes to the proposed inhibitors **12-O** and **12-N** were unambiguous control over the relative stereochemistry and regiochemistry, the avoidance of readily aromatized intermediates, and uti-

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(10) The overall charge on the complex depends on the protonation states of the incoming and departing groups.

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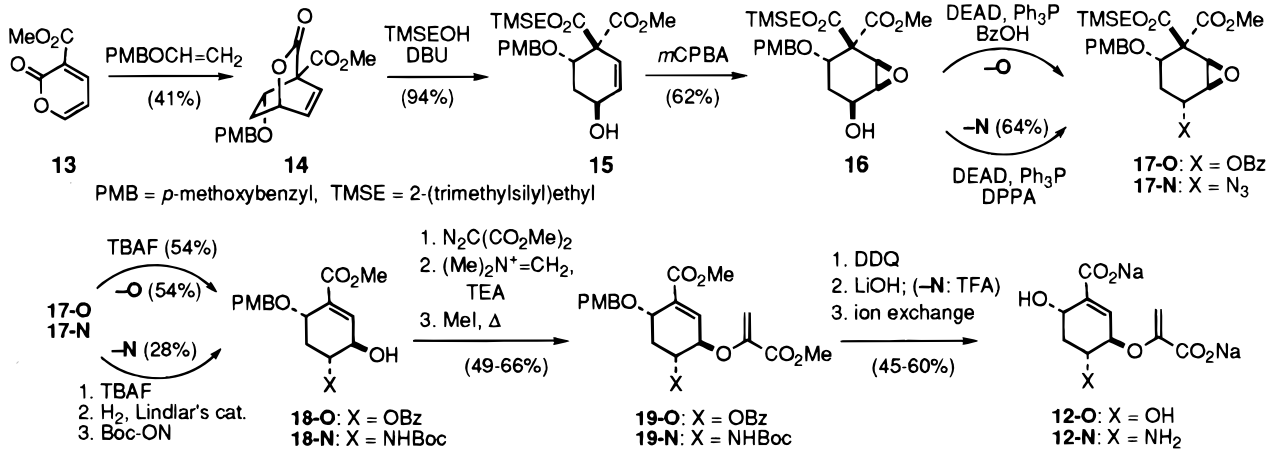
Scheme 1



lization of an advanced intermediate for both targets. The sequence shown in Scheme 1 was developed to meet these requirements.

3-Carbomethoxy-2-pyrone, **13**, has been explored as the diene component in inverse electron demand Diels–Alder reactions by Markó and Evans.[11,12] Various vinyl ethers can serve as the Diels–Alder partner, and the reaction can be carried out both thermally and with Lewis acid catalysis. Cycloaddition with *para*-methoxybenzyl vinyl ether (prepared in 44% yield over three steps from ethylene glycol[13]) provides bicyclic lactone **14** in which the hydroxyl at C-6 is protected as the PMB ether. Comparison of the NMR characteristics of lactone **14** with data reported by Markó and Evans was consistent with formation of the *endo* product.[12] The lactone can be opened by alcoholysis under basic conditions. The dimethyl ester corresponding to **15**, formed in methanolic K_2CO_3, was studied first, but the fragmentation reactions later in the synthesis (**17** to **18**) proved to be easier to carry out when the two esters can be cleaved selectively. Thus, the mixed diester **15** was prepared from 2-(trimethylsilyl)ethanol in the presence of DBU. Little, if any, product of ester exchange is formed under these conditions, even when a large excess of the alcohol is used to drive the reaction to completion over a 3-day period.

The β-oriented oxygen at C-3 was introduced through epoxidation, directed by the allylic hydroxyl group in **15**; epoxy alcohol **16** then served as the branchpoint along the synthetic routes to the two inhibitors. The configuration at the C-4 carbon in **16** was inverted using the Mitsunobu protocols.[14] Esterification with benzoic acid required excess reagents for complete conversion of the starting material to **17-O**, and purification from triphenylphosphine oxide was deferred until after the next step. For synthesis of the amine derivative, nitrogen was introduced with inversion using diphenylphosphoryl azide in the Mitsunobu process to give azide **17-N**.

Treatment of these epoxides with tetrabutylammonium fluoride (TBAF) cleaves the TMSE ester, triggering decarboxylation and epoxide opening in a Grob-type fragmentation process, to provide the conjugated allylic alcohols **18**. In the amino series, the azide is reduced and the resulting amine protected as the Boc-derivative at this stage.

The enolpyruvyl side chains were introduced according to Ganem's protocol.[15] Rhodium-catalyzed insertion of dimethyl diazomalonate into the OH bond gives the malonyl ethers, which undergo alkylation with Eschenmoser's reagent. In the oxygen series (but, interestingly, not in the nitrogen series), this adduct is prone to reversion to the malonyl ether during chromatographic purification, so both were carried on directly by exhaustive methylation and fragmentation to **19-O** and **19-N**. The PMB protecting group is cleaved from each analogue with dichlorodicyanobenzoquinone, and the esters are saponified with lithium hydroxide. The amino group of **12-N** is unveiled at the end to prevent lactam formation by cyclization onto the enolpyruvyl ester. The syntheses of **12-O** and **12-N** were thus accomplished in 10 and 13 steps, respectively, from 3-carbomethoxy-2-pyrone.

Purification of these compounds proved to be more difficult than for the previously reported isomers, **9**–**11**. Both **12-O** and **12-N** are hygroscopic and difficult to separate from the salts used in the ion exchange process and, moreover, appear to be sensitive to decomposition, e.g., by hydrolysis of the enolpyruvyl side chains. The diol has been obtained in 80% purity (based on combustion analysis), while the amino alcohol is available in only 33–40% purity (based on elemental analysis or quantitative ^{1}H NMR using lithium benzoate as an internal standard, respectively). In each case, the major impurities are inorganic salts, since they are not apparent by NMR. The biological activity of these compounds will be reported at a later date.

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Supporting Information Available: Experimental procedures and characterization for all compounds described. This material is available free of charge via the Internet at http://pubs.acs.org.

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